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RESEARCH

Selenium-Enriched *Aspergillus oryzae* A02 Enhances Testicular Antioxidant Capacity in Mice by Regulating Intestinal Microbiota and Serum Metabolite

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Abstract

Selenium (Se) is a trace element that is essential for health. Organic Se created by Se-enriched microorganisms has the characteristics of low toxicity, high bioavailability, and regulation of physiological functions. Here, the regulatory effect of Se-enriched *Aspergillus oryzae* A02 on the reproductive function of male mice and its potential molecular mechanism was studied. Specifically, twenty-four male mice were randomly divided into a control group and a Se-enriched *A. oryzae* A02 (Nano-Se) (daily gavage of 0.5 mg/kg, dissolved in saline) for an 8-week experiment. The results showed that Nano-Se intervention did not affect body weight and testicular index, but increased sperm concentration and seminiferous epithelium height in experimental mice, indicating that Nano-Se has the potential to improve the reproductive performance of male mice. Mechanistically, Nano-Se intervention increased the levels of antioxidant-related indicators catalase (CAT) and glutathione peroxidase (GSH-Px) in mouse serum, and increased the relative mRNA expression of *GSH-Px*, heme oxygenase-1 (*HO-1*), and NADPH quinine oxidoreductase-1 (*NQO-1*) in testicular tissues. We identified 9,10,13-trihydroxyoctadecenoic acids (TriHOMEs), stearidonic acid and selenomethionine linked with alpha-linolenic acid metabolism, selenocompound metabolism, folate biosynthesis, ubiquinone, and other terpenoid-quinone biosynthesis and biosynthesis of cofactors. In addition, Nano-Se did not influence the fecal bacterial alpha and beta diversity ($P > 0.05$), but increased the abundance of the Actinobacteriota and Proteobacteria phyla and the *Staphylococcus* and *Corynebacterium* genera, and lowered the abundance of the Bacteroidota phylum and the *Lactobacillus* and *norank_f_Muribaculaceae* genera. Nano-Se is considered a novel and promising nutritional regulator to improve reproductive function.

Keywords Nano-Se · Male reproduction · Microbiomics · Metabolomics · Mice

Introduction

According to the Global Burden of Disease, the global fertility rate has been steadily declining over the last 20 years. Epidemiological studies have also shown that male sperm quality has significantly deteriorated [1]. Although the factors behind the global fertility decline are unknown [2], the decline in sperm quality has become a major public health issue in reproductive and life cycle health [3]. Improving male fertility is a critical global health issue. Identifying strategies to enhance sperm quality is essential to addressing this challenge.

Spermatozoa is particularly vulnerable to oxidative stress due to their limited antioxidant capacity and repair mechanism [4, 5]. MacLeod reported as early as 1943 that sperm viability was reduced in high oxygen conditions [6]. Oxidative stress can cause lipid peroxidation,

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damage to nucleic acids, and loss of motility and reduced fusion capacity of spermatozoa [7, 8]. Oxidative stress contributes to sperm cell dysfunction and male infertility due to its negative effects on the functional and structural integrity of sperm [4, 9, 10]. Therefore, the application of antioxidant nutrients to control the sperm redox balance has become an increasing research focus.

Selenium (Se), as one of the essential trace minerals, plays crucial roles in antioxidation, immune regulation, and reproductive functions [11, 12]. Compared to the extremely narrow threshold range between the beneficial biological dosage and toxic result limit of inorganic Se, Se-enriched *A. oryzae* A02 (Nano-Se) has higher biological activity and lower toxicity than inorganic and organic non-Nano-Se compounds [13, 14]. Nano-Se has a wide range of beneficial effects, including antitumor, reproductive protection, antioxidant, hypolipidemic, and anti-inflammatory effects [15–18]. Therefore, Nano-Se is considered a novel and promising dietary supplement that may be an optimal substitute for other Se sources. However, the precise mechanism by which Nano-Se regulates sperm redox balance and enhances testicular antioxidant capacity remains unclear.

The intestinal microflora maintains normal reproductive function [19]. It has been demonstrated that the intestinal microflora can influence the reproductive capacity of animals by modulating metabolic levels. For example, mice treated with fecal microbiota transplantation have higher levels of beneficial gut bacteria, including Bacteroidetes, *Bifidobacterium*, *Sphingomonas*, and *Campylobacter*. This improves the blood metabolome and testicular metabolome, promoting spermatogenesis [20]. It also restores the gut microbial ecosystem, which can be used to treat male infertility associated with metabolic syndrome [21]. Therefore, we propose a hypothesis as to whether Nano-Se is propitious to male reproductive health by regulating intestinal microbiota and serum metabolites to improve testicular antioxidant capacity. This study was based on 16 s rDNA high-throughput sequencing, non-targeted metabolomics techniques, and the related experiments to analyze the roles and mechanisms of Nano-Se in regulating testicular function.

Materials and Methods

Reagents

Nano-Se used in this study was synthesized using Se-enriched *A. oryzae* A02. The size of Se nanoparticles in the mycelium pellets of *A. oryzae* A02 ranges from 40 to 75 nm, as confirmed by the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences [22].

Animals and Experimental Design

A total of 24 6-week-old male Balb/c mice (STA Experimental Animal Co., Ltd., Hunan, China) were used in this study. Animals were kept in the animal breeding facility of the institute at 25 ± 2 °C, 12:12-h light/dark cycle, and 60 ± 10% humidity with 2 mice per cage. The mice had free access to feed and water. After a 1-week acclimatization period, the mice were randomly divided into the control group and the Nano-Se group, and each group was repeated 6 times (one cage/time). All mice were fed with normal diets. The mice in the control group were fed with water, while mice in the Nano-Se group were fed with water containing 0.5 mg/kg●BW Nano-Se. The dose of Nano-Se in the experiment was determined by referring to the previous study [23]. The water bottle was changed every 2 days. Cages were changed once a week. Experiments were performed for 8 weeks and body weight was measured every week. The whole experimental procedure was conducted in strict compliance with the requirements of the Ethics Committee of the Institute of Subtropical Agriculture of the Chinese Academy of Science (No.ISA-2020–18).

Sample Preparation

At the end of the experiment, all animals were fasted for 12 h and feces were quickly collected for microflora analysis. Blood was collected from the eyeball and kept at room temperature for 30 min. The blood samples were centrifuged at 3000 g and 4 °C for 15 min. Then, the serums were collected and stored at − 20 °C for metabolomic analysis. After blood collection, the male mice were sacrificed by cervical dislocation, and then epididymis and testicular samples were excised, weighed, and stored at − 80 °C for further analysis. The testicular index (‰) was calculated as follows: [weight of testis pair (g)/body weight (g)] × 1000 [24].

Sperm Analysis and Testicular Histology

The sperm suspension was prepared according to the method of Amann and Lambiase [25]. Briefly, collect the cauda of the epididymis of each mouse and put them in a cell culture dish containing 4 mL of 0.9% normal saline at 37 ℃, cut them with scissors, incubate them at 37 ℃ for 20 min, release spermatozoa, and count the spermatozoa using a hemocytometer.

For testicular histological analyses, the left testes were dehydrated and embedded in paraffin. Five-micrometer-thick paraffin sections were stained with hematoxylin and eosin for histopathological evaluation. The images of stained samples were captured by a light microscope (Olympus CX22)

under 200 magnification, and the testicular morphometric method was analyzed using Image Pro Plus software23 with reference to the previous study [26].

Measurement of Antioxidant Enzymes and Testosterone

Testosterone, catalase (CAT), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) concentrations in the serum were determined by commercial assay kits according to the manufacturer's instructions. The specific information of commercial reagent kits is shown in Table 1.

qRT-PCR

The mRNA expression levels in the genes were determined by qRT-PCR method. Testicular tissues were homogenized with total RNA extraction reagent (TRIzol, reagent, Tiangen Biotechnology Company, Ltd., Beijing, China) and total RNA was extracted according to the manufacturer's instructions. The specific operation procedures refer to the method of Ginzinger [27]. A UV spectrophotometer was used to determine the concentration and purity of RNA. cDNA was synthesized by reverse transcriptase and amplified by Taq DNA polymerase. All primers used in this study were synthesized by Sangon Biotech (Shanghai, China) and are listed in Table 2.

Sequencing Data Analysis

Analysis of Intestinal Microbial Diversity

In this experimental analysis, a series of experimental procedures are required, such as fecal DNA extraction, concentration detection, purity and quality detection of DNA, and 16 s rRNA PCR amplification. For the specific test operation process, please refer to the literature published by Niu et al. [28].

Workflow of Serum Non-Target Metabonomics Analysis

Serum metabolites were measured by a commercial service facility of the Biotree Company (Shanghai, China). Briefly, serum samples from mice were collected and immediately stored at − 80 °C. For sample pretreatment, an aliquot of 50 µL serum sample was mixed with 400 µL acetonitrile-methanol (1:1, v/v). Then, the mixture was ultrasonically treated at 5 ℃ for 30 min at 40 kHz. The sample was placed at − 20 °C for 30 min to precipitate the protein. After centrifugation at 13,000 g for 15 min at 4 °C, the supernatant was carefully transferred to a new microtubule and evaporated

Table 1 Commercial reagent kit information

Name	Company	Art No
QuicKey Pro Mouse T (Testosterone) ELISA Kit	Elabscience	E-OSELM0003
Superoxide Dismutase (SOD) Activity Assay Kit	Boxbio	AKAO001M
Malondialdehyde (MDA) Content Assay Kit	Boxbio	AKFA013M
Mouse Glutathione peroxidase for research use only	MEIMIAN	MM-44117M2
Mouse Catalase for research use only	MEIMIAN	MM-44125M1

Table 2 Primers used for real-time quantitative PCR

Target gene	Accession no.[a]	Primer sequence (5′—3′)	Product (bp)
Nrf2	NM_010902.4	F: AGCGGTAGTATCAGCCA R: GCCCAGTCCCTCAATAGC	150
HO-1	NM_010442.2	F: TGTTGCGCTCTATCTCC R: GTACACATCCAAGCCGAG	136
NQO-1	NM_036153810.1	F: GGCCAATGCTGTAAACCA R: GCGGCTCCATGTACTCT	129
SOD-1	NM_011434.2	F: TGTGACTGCTGGAAAGGACG R: ACTGCGCAATCCCAATCACT	203
SOD-2	NM_013671.3	F: CAGACCTGCCTTACGACTATGG R: CTCGGTGGCGTTGAGATTGTT	113
CAT	NM_009804.2	F: CAAGATTGCCTTCTCCGGGT R: GACCCCGCGGTCATGATATT	223
GSH-Px	NM_001329528.1	F: TACACCGAGATGAACGATCTG R: ATTCTTGCCATTCTCCTGGT	102
β-actin	NM_007393.5	F: TGTCCACCTTCCAGCAGATGT R: AGCTCAGTAACAGTCCGCCTAGA	101

to dryness under mild nitrogen flow. The metabolites in the serum were analyzed and detected using ultra-high-performance liquid chromatography-mass spectrometry/mass spectrometry (UHPLC-MS/MS). Based on the different charge states of metabolites, we classify the detected metabolites into two forms: positively charged (POS) and negatively charged (NEG). The R package ropls (Version 1.6.2) performed principal component analysis (PCA) and orthogonal least partial squares discriminant analysis (OPLS-DA), and used 200 permutation test validation to evaluate the stability of the model. In addition, a Student *t*-test was performed. The selection of significantly different metabolites was determined based on the variable importance in the projection (VIP) obtained by the OPLS-DA model, the fold change value (FC), and the *P*-value of the Student *t*-test, and the metabolites with FC < 0.95 or > 1.05, VIP > 1, and $P < 0.05$ were significantly different metabolites. Differential metabolites among two groups were summarized and mapped into their biochemical pathways through metabolic enrichment and pathway analysis based on the database of Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/).

Metabolomics and 16S rRNA Multi-Group Correlation Analysis

The data were analyzed through the online platform of Majorbio Cloud platform [29]. Using hierarchical clustering analysis to analyze Spearman's correlation between bacteria and metabolites, and using heatmap packages to more directly display differences.

Statistical Analysis

Statistical analysis and testicular histological measurement were conducted using SPSS Statistics 22 software (IBM, USA) and Image-Pro-Plus 6.0 software (MEDIA CYBERNETICS, Bethesda, MD, USA) respectively. Significant differences between groups were performed on the Student *t*-test or nonparametric tests. The differences were considered significant when $P < 0.05$.

Results

Nano-Se Did Not Affect the Growth and Testicular Index

Changes in body weight and testicular index of mice are shown in Fig. 1. There were no statistically significant changes in body weight (Fig. 1A) and testicular index (Fig. 1B) compared to the control group.

Nano-Se Promoted Testicular Development

The sperm count was significantly increased in the Nano-Se group compared to the control group (Fig. 2B, $P < 0.05$). The testicular histological analysis showed that the mice testes had a clearer seminiferous tubule structure, the wall basement membrane was more complete, the spermatogenic cells were neatly arranged, and the lumen was filled with more mature sperm in the Nano-Se group compared to the control group (Fig. 2A). While seminiferous tubule lumen diameter did not show significant changes in the Nano-Se group, seminiferous epithelium height was significantly improved (Fig. 2D, E, $P < 0.05$). However, Nano-Se supplementation had no effects on serum testosterone concentration (Fig. 2C, $P \geq 0.05$).

Nano-Se Enhanced the Antioxidant Capacity of the Testes

Nano-Se supplementation improved the antioxidant capacity of mouse testicular tissue. GSH-Px concentration was significantly increased in the Nano-Se group (Fig. 3B, $P < 0.05$), and there was a trend of increased CAT content (Fig. 3A,

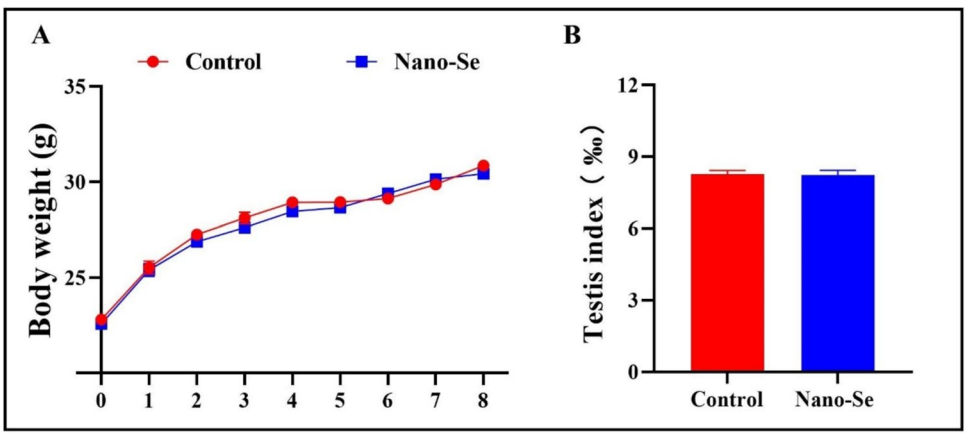


Fig. 1 Effects of Nano-Se on growth performance in male mice. **A** Body weight. **B** Testicular index. Values indicate the mean ± SEM ($n = 10$)

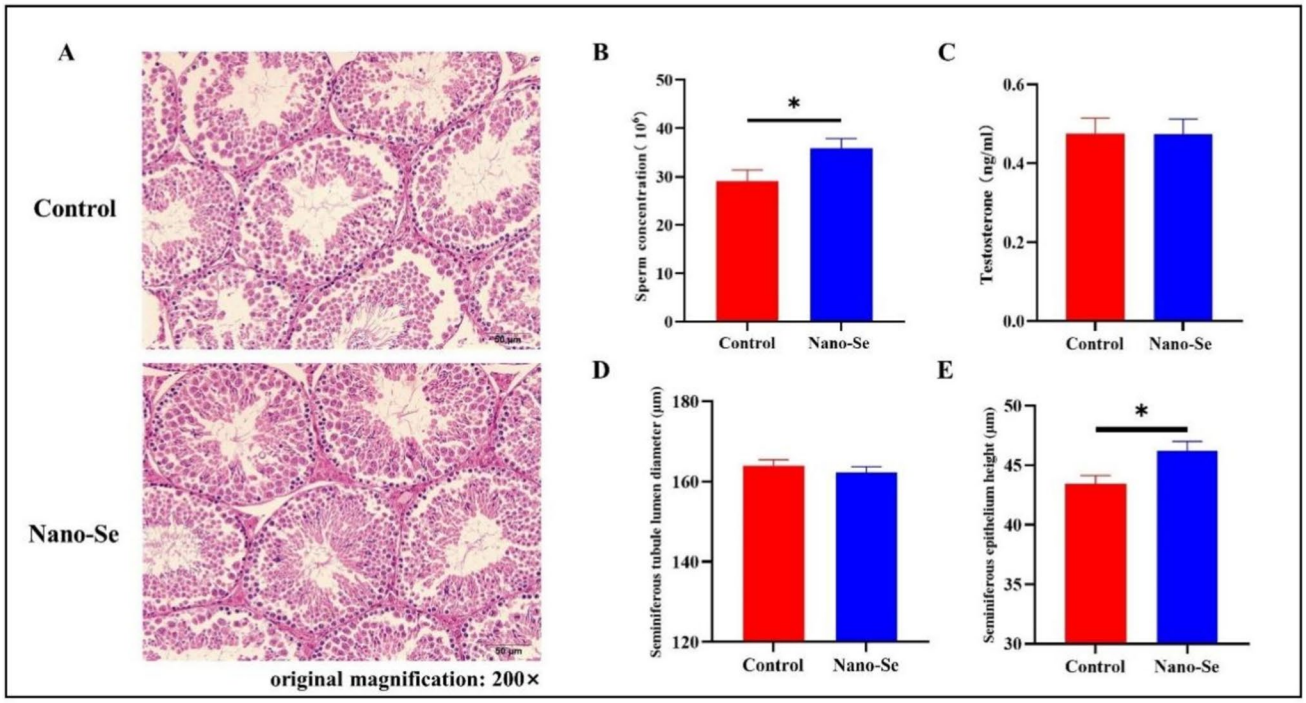


Fig. 2 Effect of Nano-Se on testicular development in male mice. **A** Testicular histological of the control group and the Nano-Se group, **B** sperm concentration ($n=10$), **C** serum testosterone ($n=6$), **D** seminiferous tubule lumen diameter ($n=50$), and (**E**) seminiferous epithelium height ($n=50$). Values indicate the mean ± SEM. *Significant difference at $P<0.05$ level

$P=0.075$), while the testicular MDA and SOD were not affected by Nano-Se supplementation (Fig. 3C and D). We also determined the effect of Nano-Se supplementation on the testicular gene expression of the above-mentioned aspects. The results showed that Nano-Se significantly upregulated *HO-1* and *NQO-1* (Fig. 4E, $P<0.05$) and GSH-Px gene expressions (Fig. 4F, $P<0.01$) compared with the controls. However, Nano-Se supplementation had no effects on nuclear factor erythroid-2-related factor 2 (*Nrf2*), SOD-1, SOD-2, and CAT gene expressions (Fig. 4E, $P\geq0.05$).

Nano-Se Changed Intestinal Microbial Diversity

16S ribosomal RNA (rRNA) gene sequencing technology was used to study the effect of Nano-Se on the diversity and composition of fecal microbiota in male mice. Based on the observed species and Chao α-diversity indices, Nano-Se did not significantly alter the alpha diversity of fecal microbes (Fig. 4A). The beta diversity was analyzed using an unweighted unimodal matrix for PCA and PCoA plots, but it did not show significantly isolated fecal microbiota (Fig. 4B). Notably, 479 core species were identified, including 231 in the control group and 184 in the Nano-Se group (Fig. 4C). The dominant phyla were Firmicutes, Actinobacteriota, Bacteroidetes, Proteobacteria, and Bacteroidota (Fig. 4D), and the dominant genera were *Lactobacillus*, *Staphylococcus*, *Corynebacterium*, *norank_f_Muribaculaceae*, and *Bifidobacterium* (Fig. 4E). Of these, Nano-Se significantly enriched the abundance of Actinobacteriota and Proteobacteria phyla and the genera *Staphylococcus* and *Corynebacterium* while reducing the abundance of Bacteroidota phylum and the genera *Lactobacillus* and *norank_f_Muribaculaceae*. At the phylum level, we did not find significant changes in bacteria. However, at the genus level, compared with the control group, Nano-Se led to significant increases in the levels of *unclassified_f__Enterobacteriaceae* and decreases in the level of *Enteractinococcus*, *Dubosiella*, *unclassified_f_Planococcaceae*, *Flaviflexus*, and *Stenotrophomonas* (Fig. 4F).

Nano-Se Changed the Composition of Serum Metabolites

Untargeted metabolomics methods were used to analyze the effects of Nano-Se on serum metabolites in male mice. With the PCA and OPLS-DA results, the metabolite profile datasets were clustered separately between Nano-Se and the control groups (Fig. 5A and B). The permutation test showed that the OPLS-DA model was reliable without overfitting (Fig. 5C). The volcano plot shows significant changes in the serum metabolite profiles for the Nano-Se group. Overall, 25 metabolites were upregulated and 60

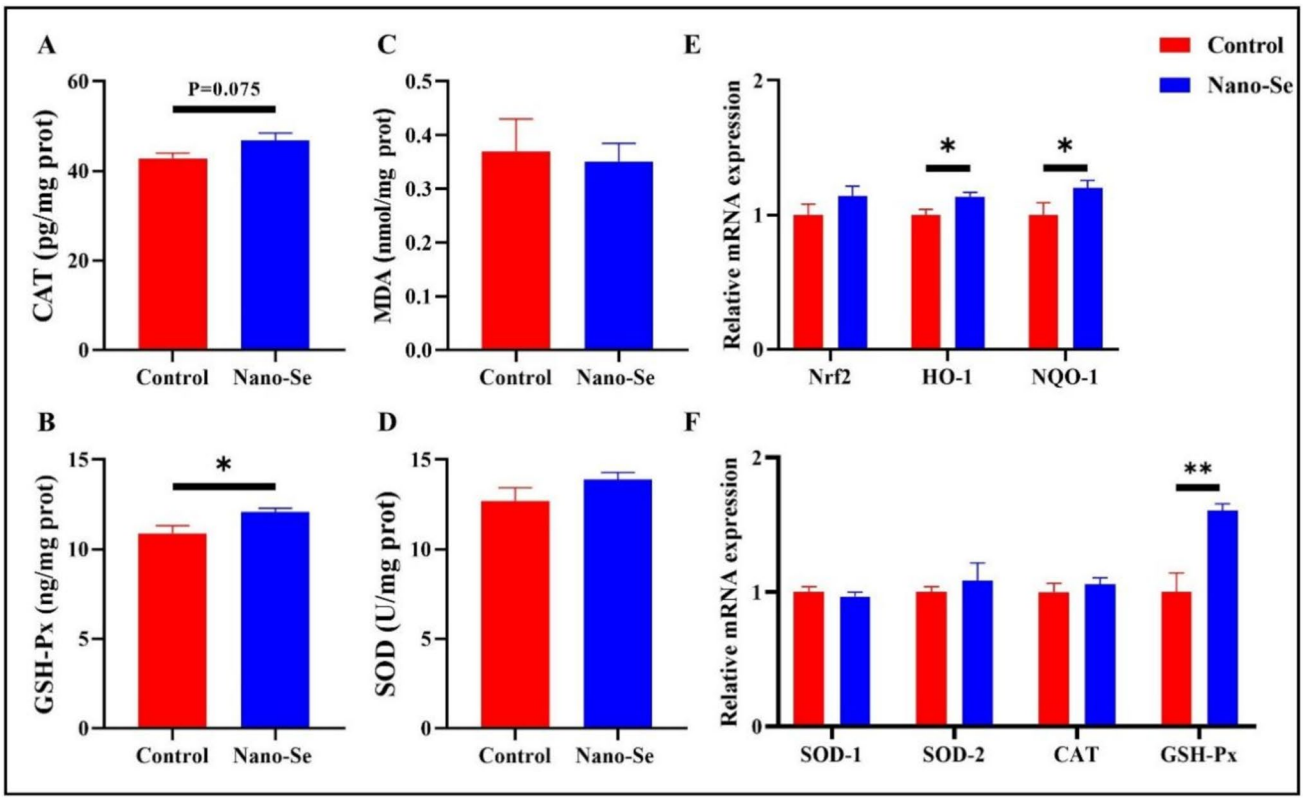


Fig. 3 Effects of Nano-Se on antioxidant capacity of testes in male mice. The content of (**A**) CAT, **B** GSH-Px, and (**C**) MDA and the activities of (**D**) SOD in the testicles. The gene expression of (**E**) the factors involved in the Nrf2 signaling pathway and (**F**) testicular antioxidases in the testicles. Values represent the mean ± SEM ($n = 5$–6). *Significant difference at $P < 0.05$ level. **Significant difference at $P < 0.01$ level

were downregulated compared with those in the control group (Fig. 2D). Using the OPLS-DA model, 79 discriminative biomarker metabolites with an initial VIP value > 1 and *P*-value < 0.05 were selected. Subsequently, 29 metabolites were selected based on whether the fold change of metabolites was < 0.95 or > 1.05 (Fig. 5E). Specifically, among these metabolites, selenomethionine, mulberrin, 8,11-eicosadiynoic acid, pregnanetriolone, and 3a,7a,12b-trihydroxy-5b-cholanoic acid were upregulated, while glutaraldehyde, pyrogallol-1-O-sulfate, cycloalliin, P-salicylic acid, stearidonic acid, 3-formyl-6-hydroxyindole, resolvin E1, sambutoxin, and (S)C(S)S–S-methylcysteine sulfoxide were downregulated in the Nano-Se group (Fig. 5E). Notably, four of these discriminant metabolites who were P-salicylic acid, selenomethionine, stearidonic acid, and 9,10,13-triHOME were associated with metabolic pathways encompassing alpha-linolenic acid metabolism, linoleic acid metabolism, selenocompound metabolism, folate biosynthesis, ubiquinone, and other terpenoid-quinone biosynthesis and biosynthesis of cofactors (Fig. 5F). And the metabolic pathways of alpha-linolenic acid metabolism, linoleic acid metabolism, and selenocompound metabolism showed significant changes (Fig. 5F, $P < 0.05$). Other metabolites have no corresponding metabolic pathway in KEGG database (http://www.kegg.jp/kegg/pathway.html).

Correlation Study of Nano-Se on Serum Metabolites and Intestinal Microorganisms

Based on the serum metabolite and 16S rRNA sequencing results, Spearman's correlation analysis was used to explore the correlation between the 6 changed bacteria and 29 differentially altered serum metabolites (Fig. 6). Among the bacteria, *unclassified_f__Enterobacteriaceae*, *unclassified_f__Planococcaceae*, *Flaviflexus*, *Dubosiella*, *Enteractinococcus*, and *Stenotrophomonas* were strongly correlated with the different metabolites. The *unclassified_f__Enterobacteriaceae* genus showed positive correlation with stearidonic acid, P-salicylic acid, and 9,10,13-TriHOME, but negative correlation with selenomethionine. The *unclassified_f_Planococcaceae*, *Flaviflexus*, *Dubosiella*, *Enteractinococcus*, and *Stenotrophomonas* showed positive correlation with selenomethionine. It was negatively correlated with stearidonic acid, P-salicylic acid, and 9,10,13-TriHOME. Taken together, the results showed

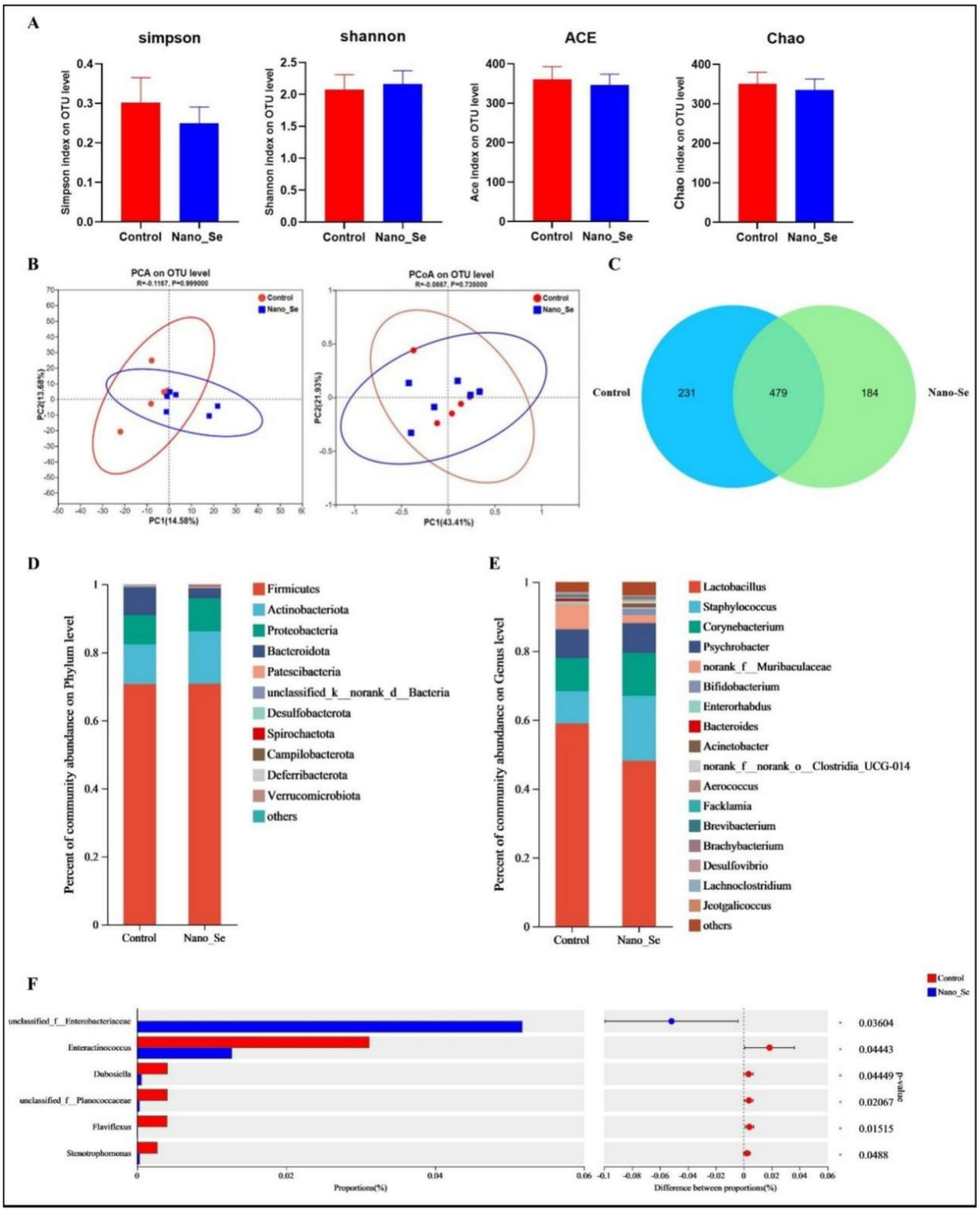
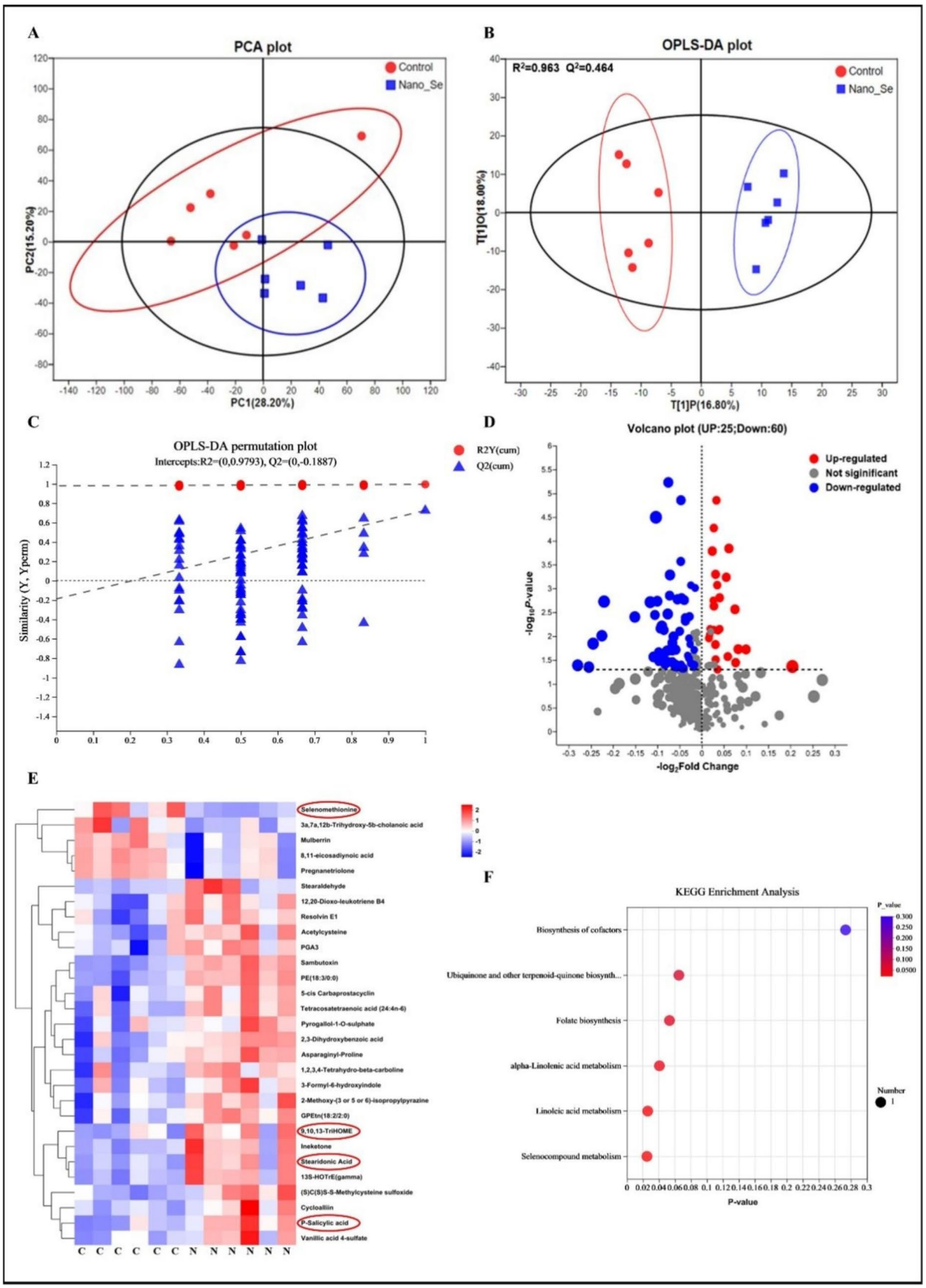


Fig. 4 Effects of Nano-Se on intestinal microflora in mice. **A** Alpha diversity indexes. **B** Beta diversity indexes. **C** Venn diagram of the sample OTU. **D** Phylum level. **E** Genus level. **F** Student's *t*-test bar plot on Genus level. *Significant difference at $P < 0.05$ level


A
PCA plot
Control
Nano_Se
PC2(15.20%)
PC1(28.20%)
B
OPLS-DA plot
R²=0.963 Q²=0.464
Control
Nano_Se
T[1]O(18.00%)
T[1]P(16.80%)
C
OPLS-DA permutation plot
Intercepts:R2=(0,0.9793), Q2=(0,-0.1887)
R2Y(cum)
Q2(cum)
Similarity (Y, Ypcrm)
D
Volcano plot (UP:25;Down:60)
Up-regulated
Not siginificant
Down-regulated
-log10P-value
-log2Fold Change
E
Selenomethionine
3a,7a,12b-Trihydroxy-5b-cholanoic acid
Mulberrin
8,11-eicosadiynoic acid
Pregnanetriolone
Stearaldehyde
12,20-Dioxo-leukotriene B4
Resolvin E1
Acetylcysteine
PGA3
Sambutoxin
PE(18:3/0:0)
5-cis Carbaprostacyclin
Tetracosatetraenoic acid (24:4n-6)
Pyrogallol-1-O-sulphate
2,3-Dihydroxybenzoic acid
Asparaginyl-Proline
1,2,3,4-Tetrahydro-beta-carboline
3-Formyl-6-hydroxyindole
2-Methoxy-(3 or 5 or 6)-isopropylpyrazine
GPEtn(18:2/2:0)
9,10,13-TriHOME
Ineketone
Stearidonic Acid
13S-HOTrE(gamma)
(S)C(S)S-S-Methylcysteine sulfoxide
Cycloalliin
P-Salicylic acid
Vanillic acid 4-sulfate
C C C C C C N N N N N N
F
KEGG Enrichment Analysis
Biosynthesis of cofactors
Ubiquinone and other terpenoid-quinone biosynth...
Folate biosynthesis
alpha-Linolenic acid metabolism
Linoleic acid metabolism
Selenocompound metabolism
P-value
P_value
Number

◂**Fig. 5** Changes in serum metabolites in male mice treated with Nano-Se. **A** Principal component analysis PCA plot, **B** orthogonal partial least squares discriminant analysis OPLS-DA score plot, **C** OPLS-DA permutation test plot and (**D**) Volcano plot, **E** heatmap of hierarchical clustering analysis, and (**F**) KEGG enrichment pathway analysis (control group vs Nano-Se group)

that the changing bacterial structure could impact the composition of serum metabolite constituents.

Discussion

The current shift in lifestyle is contributing to a gradual increase in male infertility, which is linked to oxidative stress caused by factors such as obesity and diseases [30, 31] This trend is becoming a significant public health concern. The testis has been extensively studied as the principal male reproductive organ [32]. Its functions encompass testicular development, spermatogenesis, and sperm maturation The effects of Nano-Se on male fertility have been widely documented [33, 34], with the antioxidant capacity of Nano-Se representing a significant focus of research [35–37]. Furthermore, the study investigated the effects of Se supplementation (including sodium selenite, Se yeast, nano-selenium) in animals. Their findings indicated that Se yeast can be an effective source of Se supplementation, which enhances antioxidant capacity [38]. Subsequent studies revealed that supplementation of hens with Nano-Se can enhance antioxidant capacity and that Se supplementation is more effective [39]. Based on these findings, the present experiment directly investigated the reproductive performance of Nano-Se synthesized from Se-enriched *A. oryzae* A02 in male mice [22]. The results demonstrated that Nano-Se favored testicular development in terms of sperm and seminiferous tubule quality. The impact of Nano-Se treatment on body weight has been extensively documented in previous studies. Rehman et al. demonstrated that Nano-Se supplementation enhanced body weight in broiler chickens [40]. However, no notable influence of Nano-Se on body weight and associated organ indices was observed in diabetic mice [41]. In the present study, the authors evaluated the impact of Nano-Se treatment on the growth performance of mice. They similarly found no significant effect of Nano-Se on body weight and testicular indices. Differences in the results of the above-mentioned studies may be related to the differences in the respective experimental design, the dose, the Se characteristics, and the experimental environment. In rats, Nano-Se was also observed to enhance antioxidant capacity, as evidenced by the upregulation of GSH-Px levels and the expression of GSH-Px, HO-1, and NQO-1 genes [36]. This indicates that Nano-Se may increase sperm count and promote testicular development in male mice by enhancing antioxidant capacity. The capacity of Nano-Se to enhance reproductive performance by upregulating antioxidant capacity has been demonstrated not only in testes [36] but also in oocytes [33].

Although Nano-Se has been extensively studied in reproduction, the combined analysis of intestinal flora and serum metabolites in normal mice in the Nano-Se group has been less studied. Prior research has demonstrated that a balanced intestinal flora is crucial for the maintenance of normal reproductive function [19, 21]. Nano-Se has been demonstrated to enhance the intestinal microbial diversity, thereby maintaining and improving overall intestinal ecology [42]. It has been reported that Nano-Se can normalize the gut microbiota imbalance induced by a high-fat diet in juvenile carp fish by increasing the abundance of beneficial bacteria, such as *Fusarium* (a fungus) [43]. Additionally, Nano-Se has been shown to alleviate the intestinal damage caused by heat stress by regulating the activity of intestinal microorganisms [44]. In this study, 16S rRNA sequencing was employed to investigate the modulatory effects of Nano-Se on the intestinal flora of male mice. The results of 16S rRNA sequencing were mainly reflected in the changes of the composition of intestinal microflora at the genus levels. For example, Nano-Se significantly increased the abundance of the Actinobacteriota and Proteobacteria phylum and the genera abundance of *Staphylococcus* and *Corynebacterium* whereas it decreased the abundance of Bacteroidota phylum and the genera *Lactobacillus* and *norank_f_Muribaculaceae*. 2,6-Dimethoxyterephthalic acid and 1-hydroxy-nonascorbic acid produced by Actinobacteriota actinomycetemcomitans quench hydrogen peroxide and cationic radicals [45], while Bacteroidota has also been linked to oxidative stress [46]. These studies also lend support to the notion that nano-selenium can enhance the diversity of gut microbes, thereby modulating oxidative stress.

It is well known that metabolic homeostasis plays a very critical role in spermatogenesis [47, 48]. In particular, lipid metabolism balance is extremely important for male reproductive health. Dysregulation of lipid metabolism increases the level of oxidative stress in testicular tissue, leading to male infertility [49, 50]. In this study, 6 metabolite pathways were identified by non-target serum metabolomics. It is worth noting that alpha-linolenic acid metabolism, linoleic acid metabolism, and selenocompound metabolism are closely related to reproductive function [51–56]. Related differential metabolites include 9,10,13-trihydroxy-octadecenoic acids (TriHOMEs), stearidonic acid, and selenomethionine. In the present study, the authors observed that the metabolism of 9,10,13-TriHOME is upregulated, which is the primary end product of linoleic acid oxidation [51] and can aggregate neutrophils as an important inflammatory mediator [52]. A deficiency of 9,10,13-TriHOME in the body may indicate elevated levels of inflammation [54], and the results of this study suggest that Nano-Se may

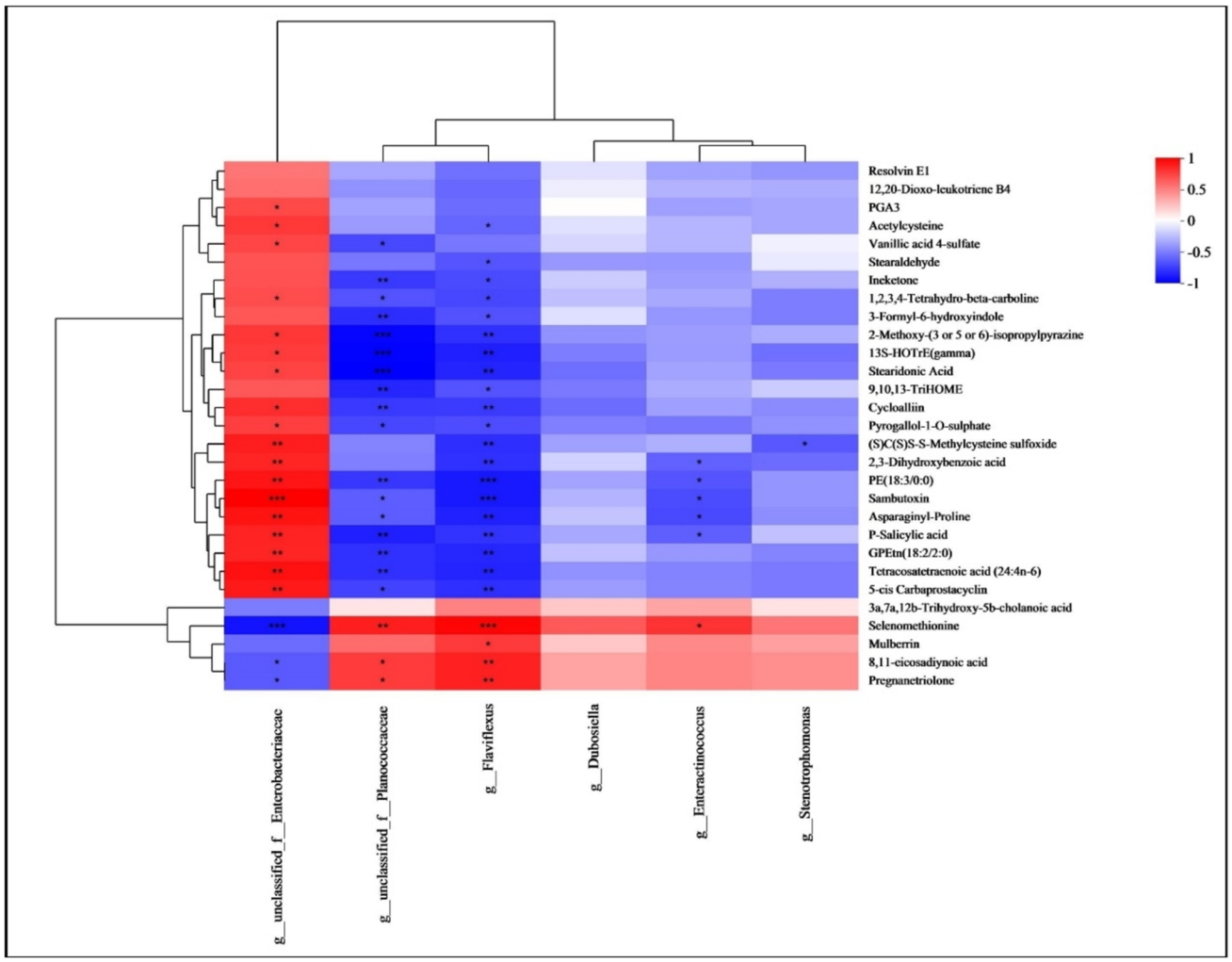


Fig. 6 The correlation analysis between the blood metabolome and the intestinal microbiota. Correlation analysis between top 6 gut microbiota and top 29 differential pos metabolites. The value is the correlation coefficient between metabolite and intestinal microorganisms. The larger the value, the stronger the correlation between the two samples. ***The correlation test $P > 0.8$, **the correlation test $P > 0.7$, and * the correlation test $P > 0.6$

possess anti-inflammatory properties. Selenomethionine metabolism, a principal intermediate in the metabolic pathway of selenium compounds [55], has been demonstrated to enhance spermatogenesis and improve reproductive performance and resistance to oxidative stress [56]. This is achieved by upregulating the Nrf2 signaling pathway [57], which leads to increased expression of antioxidant enzymes such as heme oxygenase-1 (HO-1) and NADPH quinone oxidoreductase 1 (NQO-1). Consistent with these findings, the current study demonstrated that Nano-Se treatment upregulated the expression of HO-1 and NQO-1, reduced malondialdehyde (MDA) levels, and improved testicular function. Furthermore, selenomethionine has been demonstrated to elevate GSH-Px levels by increasing the synthesis of selenocysteine, the active center of GSH-Px [58]. This result provides additional support for the finding that Nano-Se elevated GSH-Px in the present experiment. The present study demonstrated that stearidonic acid metabolism was up-regulated, which is typically considered the end product of α-linolenic acid (ALA) metabolism. ALA is a crucial n-3 polyunsaturated fatty acid (PUFA) with a range of physiological functions, including the prevention of atherosclerosis, cardiovascular and cerebral vascular diseases, regulation of blood lipids, reduction of obesity, and the promotion of positive anti-inflammatory and anti-allergic effects [59–61]. It is noteworthy that Nano-Se has been demonstrated to regulate ALA, which is essential for the promotion of testosterone biosynthesis in animals [62]. The results of non-target serum metabolomic analysis demonstrated that Nano-Se enhances antioxidant capacity and improves testicular function by modulating lipid homeostasis and selenomethionine metabolism.

It has recently been reported that metabolomic changes in fecal samples are associated with the gut microbiota in the development of diseases such as Crohn's disease [63] and diabetes [64]. In this study, 6 microbial genera and 29 different metabolites were analyzed and the relationship between specific bacteria and specific metabolites affected by Nano-Se was revealed. The key strains of these metabolism/functional metabolisms were screened. Although a definitive causal relationship could not be established through this analysis, a correlation between the two variables was identified. In this study, it was found that regulation of lipid metabolism and selenium compound metabolism by certain specific bacterial groups could improve the antioxidant capacity of testicular tissue and promote testicular function. The results of this study will lay a solid theoretical basis for further Nano-Se research. Therefore, we focus on the reproductive effects of significant changes in *unclassified_f__Enterobacteriaceae*, *Enteractinococcus*, *Dubosiella*, *Flaviflexus*, *unclassified_f__Planococcaceae*, and *Stenotrophomonas* after Nano-Se treatment, in order to explore the correlation between intestinal bacteria and specific metabolites more deeply.

Conclusion

In conclusion, this study demonstrates the potential of Se-enriched *Aspergillus oryzae* A02 (Nano-Se) as a dietary supplement to enhance male reproductive health. By modulating the gut microbiota and altering serum metabolite profiles, Nano-Se can improve antioxidant capacity and testicular function. These findings provide valuable insights into the mechanisms underlying the beneficial effects of selenium supplementation on male reproductive health.

Author Contribution J Yang and Y Li contributed to the study conception and design. Animal experiment, sample preparation and analysis, data collection and processing, and statistical analysis were performed by J Du, X Tian, J Luo and Ali Doğan Ömür. The first draft of the manuscript was written by J Du, Y Li, J Yang and J Xue revised the manuscript. All authors read and approved the final manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of Interest The authors declare no competing interests.

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